membranes as well as facilitates the formation of lipid membrane by an attractive electrostatic interaction between SUVs and gels.We expect these gel supports to allow us to prepare a lipid membrane from a proteoliposome. Since the chemical composition of a gel is easily modified, we can obtain a gel array with desirable properties such as mechanical strength, electric charge, and responsiveness to a stimulus.

3150-Pos Board B842

Parallel Reconstitution of Bacterial Toxins, Porins and Ion Channels into Suspended Lipid Membrane Microarrays for High-Throughput Electrophysiology

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High-throughput electrophysiological recordings in artificial membranes have a great potential for pharmaceutical screening and nanopore analytics. In addition to parallelization and automation of lipid bilayers formation, different protein reconstitution protocols are required depending on the concrete application.

We report on functional reconstitutions of three classes of pore-forming proteins into suspended lipid bilayers on Micro Electrode Cavity Array (MECA). Three reconstitution strategies have been employed and optimized depending on the protein structure: self-insertion for pore-forming toxins, transfer from detergent micelles for porins and vesicle fusion for ion channels.

By optimizing protein concentration and facilitating spontaneous insertion with short voltage pulses just below the electroporation threshold, a controlled insertion of alpha-hemolysin was achieved. Exactly one pore per bilayer was inserted in over 50% of bilayers in the array, which is substantially higher than the 37% predicted by the Poisson distribution.

We investigated and optimized insertion of outer membrane porins OmpF, MspA and OccK1 into bilayer arrays via detergent dilution. Best strategy was to use mild nonionic detergents and sequentially dilute protein stock solution to minimize influence of the detergent on the membrane. Protein injection in the immediate vicinity of a bilayer array and thorough mixing of the solution were decisive factors for homogeneous distribution of functional porins.

Reconstitution via detergent micelles was found to be problematic for ion channels, but proteovesicle fusion was successful. The tetrameric potassium channel KcsA was expressed *in vitro* with cotranslational insertion into lipid vesicles or nanodiscs. Transfer from nanodiscs into suspended bilayers was shown to be inefficient. Additionally, the presence of nanodiscs substantially destabilized the membranes. For proteovesicles best fusion rates were achieved upon addition of negatively charged phospholipids followed by extrusion of the recovered proteovesicles through a polycarbonate filter.

3151-Pos Board B843

A Miniaturized Single Channel Amplifier for Various Different Electrophysiology Setup

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Different electrophysiology setups allow single ion channel recordings using Lipid Bilayer Membrane (BLM) for protein insertion. Ion channels are transmembrane proteins involved in many biological process of cell life. They are used as target for drug discovery and single-molecule sensors. Synthetic nanopores can also be used as nano-coulter counters and very promising transducers for DNA sequencing. Complex protocols are under investigation to assembly ion channels on reconstituted lipid membranes. They use different setups, ranging from suspended BLM, microfluidic and lab-on-a-chip devices, planar patch clamp, tip-dip method. These studies, combined to new emerging nanopore applications, require high sensitive and high resolution instrumentation, able to detect small currents (in the pA range) over large bandwith (up to 100kHz), providing flexible voltage stimuli generation. We present a compact and complete system for single ion channels and synthetic nanopores experiment, useful for scientists and students for academic purposes, composed by:

- a miniaturized low noise single channel voltage-clamp amplifier and digitizer integrated into a single ASIC, with direct connection to a computer USB port;
- a software interface for real time data display and analysis featuring flexible voltage stimuli control;
- customised electrode interfaces for various different electrophysiology platforms and microfluidic devices.

As a proof of concept, we demonstrate the system performances using different test proteins (KcsA, Gramicidin, Alpha-hemolysin) embedded into reconstituted lipid membranes. The acquired data are presented.

3152-Pos Board B844

Design Principles for Nanoparticles Enveloped by a Polymer-Tethered Lipid Membrane Coat

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Nanoparticles enveloped by a lipid membrane, which is anchored to the particle by means of polymer tethers, are potentially valuable drug delivery vehicles, for instance due to their enhanced stability, monodispersity, and their wide range of functionalization possibilities. While both the creation of liposomes and that of membranes tethered to flat substrates is fairly well understood, the combined task of enclosing a nanoparticle inside a liposome that is anchored by flexible polymer tethers poses a number of design challenges. These will constrain the range within which typical parameters (such as nanoparticle size, polymer length, or tethering density) can be varied. For instance, the anchoring density of tethered lipids also enforces lower bounds for the tethering density on the nanoparticle or, alternatively, its size. Here we use a combination of polymer theory and geometric constraints to derive design criteria for such systems. These can for instance be used to control the size of the coated nanoparticle by a suitable choice of tethering density and degree of polymerization. These predictions are validated by coarse-grained simulations of coated nanoparticle constructs. We also extend these simulations to characterize the mechanisms by which a lipid envelop forms around polymer-coated nanoparticles, thereby providing valuable information about conceivable processes by which such particles could be experimentally produced.

3153-Pos Board B845

Nanoparticle Layer Formation, Structure and Interactions with Model Membranes

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Nanoparticles are of great scientific interest because of the unique properties brought about by their large surface area. In bioengineering, they are used in protein engineering, drug delivery, implants and in interactions with lipid membranes. Langmuir-Blodgett and Langmuir-Schaefer deposition techniques have proven to be effective ways for controlled deposition of one molecule thick layers of nanoparticles and -rods [1-3].

Nanoparticle deposition onto a solid or liquid surface is controlled by particle-particle interactions. Here, we discuss how stabilization of nanoparticle Langmuir films can be done by using different ligand molecules or by modifying the chemical composition of the subphase. Interactions between nanoparticles and model membranes are presented. Different types of nanoparticles, including carbon nanotubes, graphene and functional nanomaterials and are discussed. Furthermore, it is shown how characterization methods such as Brewster Angle Microscopy and Polarization-Modulation Infrared Spectroscopy at the air-water interface can be used to evaluate the formation and evolution of these layers.

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3154-Pos Board B846

Cellular Binding of Charged Nanoparticle-Protein Complexes

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Nanoparticles used for biomedical applications are exposed to a complex mixture of extracellular serum proteins that non-specifically adsorb to the nanoparticle surface. The resulting "protein corona" dominates interactions of the nanoparticle with the cellular environment. As nanoparticle binding to the cell surface is the first step in the course of most biomedical applications, we have focused on the role of the initial nanoparticle surface charge in the cellular binding of nanoparticles. Cationic, amine-modified and anionic,

carboxylate-modified polystyrene nanoparticles were studied as a model system. Although both cationic and anionic nanoparticles form a protein corona, cellular binding follows opposite trends as determined from fluorescence microscopy experiments. The cellular binding of cationic nanoparticles is enhanced in the presence of serum proteins, while anionic nanoparticle binding is inhibited by the presence of serum proteins. Competition assays performed with flow cytometry enabled us to identify the cellular receptors used by the nanoparticle-protein complexes. We have determined that complexes formed with anionic nanoparticles bind to native protein receptors, while those formed with cationic nanoparticles bind to scavenger receptors. Similar trends were observed for anionic nanoparticles with biomedical applications including quantum dots, Au nanospheres, and low-density lipoprotein. To probe the underlying cause for the charge-dependent differences in cellular binding, we are currently characterizing the structure of the adsorbed proteins using circular dichroism, fluorescence spectroscopy, and isothermal titration calorimetry. Preliminary results suggest that protein structure is lost upon binding to cationic nanoparticles, and protein structure is retained on anionic nanoparticle surfaces. Our results indicate that, in the presence of blood serum proteins, initial nanoparticle surface charge mediates nanoparticle-cell interactions.

3155-Pos Board B847

Probing Osteoarthritis Biomarkers with Magnetic Nanoparticles

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Osteoarthritis (OA), a degenerative joint disease, is a large and quickly growing socioeconomic burden. Disease-modifying drugs are not available for symptomatic OA, and detection of early, asymptomatic OA presents a significant challenge. Molecular biomarkers found in the joint's synovial fluid (SF) have demonstrated a significant potential for early OA detection. However, obtaining this viscous fluid from a joint is difficult and the procedure is not always successful.

Our new technology allows collection of OA biomarkers directly from SF without the need to remove any portion of it. The technique utilizes unique magnetic properties of the superparamagnetic iron oxide nanoparticles (SPIONs). In the absence of an external magnetic field, SPIONs do not retain stable magnetization; however when a high-gradient magnetic field is applied, SPIONs experience a translational force directed towards the field source. SPION-based materials are safe and efficient. They have been approved by the FDA for use as contrast agents in MRI.

Our technology uses SPIONs embedded into polymer cores. An antibody against an OA biomarker is conjugated to the surface of the core. When particles are injected in SF, they bind OA biomarkers. The biomarker-loaded particles are then collected with a small Nd-Fe-B magnetic probe. After collection, the particles are released from the probe and the amounts of collected biomarker and particles are determined. Then initial concentration of biomarker and its total amount in SF are estimated using the magnetic collection results.

For the proof-of-concept, a widely-investigated OA biomarker, C-telopeptide of type II collagen (CTXII) was used. The stages of magnetic collection including antibody-biomarker binding, magnetic collection efficiency, biomarker and particle release techniques, and biomarker and particle quantification, are characterized in detail. Thereby we demonstrate the potential of our technolgy to serve as an enabling technology for early OA diagnosis.

3156-Pos Board B848

Magnetic Nanoparticle Delivery System for Mucus Layer Penetration

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Excessive mucus production is a common and significant problem for several prominent human lung diseases such as asthma and chronic obstructive pulmonary disease (COPD). In these diseases, the mucus barrier poses a significant challenge to drug delivery, leading to increased healthcare cost and poor quality of life for patients. One method of overcoming structural and fluid resistance of mucus is by utilizing a targeted delivery method of magnetic nanoparticles (NPs) to diseased epithelial cells. However, previous experimental studies showed no mucus penetration using superparamagnetic iron oxide (Fe_3O_4) FeNPs. In our experiments we generated a field gradient that is an order of magnitude stronger than that in previously applied FeNP delivery systems. In our delivery system, FeNPs successfully penetrated the approximately 100 μm thick mucus layer of air-liquid interface cultured primary normal human tracheobronchial epithelial cells indicating the potential of magnetic nanoparticles for targeted drug delivery to the airway mucosal surface.

3157-Pos Board B849

Gate Control of Mesoporous Silica with α-Synuclein-Coated Au Nanoparticles via Particles-On-A-Particle Assembly

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Efficient encapsulation, targeted delivery, and controlled release of drugs are critical prerequisites in current efforts to design advanced drug delivery systems. In particular, the development of release-controllable delivery systems is of importance to curb a substantial risk of systemic side effects and greatly enhance pharmacological effect of drugs at the desired target. In this study, we have developed a novel mesoporous silica-based delivery system in which the function of protein ligand-responsive gate control was embedded. The nanocomposite, named 'raspberry-type PoP', was constructed by incorporating αSyn-functionalized AuNPs onto the surface of a mesoporous silica nanoparticle (MSN) via acidic pH-induced noncovalent interaction of αSyn on hydrophilic surface, which provides controlled release behavior of its entrapped cargo mediated by ligand interaction to the protein. The PoP on which 5 nm AuNP was incorported could retain about 30% of pre-soaked Rh6G within MSN by blocking leaking-out of the dye, and the percentage was dramatically decreased as AuNP size increased. Thus, by tuning sizes of pore and/or surface nanoparticles, one can improve the efficiency of cargo entrapment. Because of human-originated α-Syn on the surface of the PoP, non-specific adsorption of plasma proteins that enhance renal and immunological clearance of intravenously-injected drug carriers might be prevented. The PoP released its entrapped dye in response to interaction of various ions with αSyn. Particularly, Ca^{2+} whose cytoplasmic concentration is tightly controlled could play as a key to open the AuNP-αSyn gate, suggesting the potential application of the PoP delivery system in Ca^{2+}-involved diseases such as cancers and cardiovascular diseases.

3158-Pos Board B850

Controlled Solid-State Synthesis of Mri Effective Superparamagnetic Maghemite Nanoparticles from Iron(II) Acetate

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Thermally induced oxidative decomposition of iron(II) acetate in air is presented as a simple route towards superparamagnetic maghemite nanoparticles the size of which can be controlled by the reaction temperature in the range of 320-400°C. Decomposition mechanism, the phase composition of the reaction products as well as their structural, magnetic and surface properties were monitored by Mössbauer spectroscopy, XRD, TEM, magnetic and BET surface area measurements. Mössbauer spectra confirm maghemite (gamma-Fe_2O_3) as the only crystalline decomposition product without any traces of other Fe_2O_3 polymorphs or magnetite (Fe_3O_4). On the other hand, the amorphous phase was identified in all samples, which is manifested by the presence of the sextet component with significantly reduced hyperfine magnetic field in the low temperature and in-field Mössbauer spectra. Its content decreases with temperature of synthesis, in accordance with increasing saturation magnetization of the samples. The size of maghemite particles can be controlled by reaction temperature from 4-8 nm at 320 °C to 20-30 nm at 400 °C. This controlled growth is clearly observed by TEM and indirectly demonstrated by narrowing of XRD lines, decreasing surface area (from 147 to 51 m^2/g) and increasing blocking and irreversibility temperatures in the FC-ZFC curves. The low intensities of the 2nd and 5th spectral lines in the in-field (5K/5T) Mössbauer spectrum of the sample prepared at 400 °C indicate a low degree of spin frustration of maghemite phase, while the amorphous admixture reveals speromagnetic behavior with unchanged intensities of spectral lines compared to the zero-field spectrum. The sample synthesized at 400 °C exhibits the MRI contrast properties fully comparable with the commercial agent Lumirem as proved by phantom experiments.